Methylenetetrahydrofolate Reductase, Alcohol Dehydrogenase, Diet, and Risk of Colorectal Adenomas[1]

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Abstract

An increased occurrence of colorectal cancer and its adenoma precursor is observed among individuals with low intakes or circulating levels of folate, especially if alcohol intake is high, although results have not been statistically significant in all studies. We examined folate and alcohol intake and genetic polymorphisms in *methylenetetrahydrofolate reductase* [*MTHFR* 667→*T* (ala→val) and *MTHFR 1298A→C* (gln→ala)] (associated with reduced MTHFR activity) and in *alcohol dehydrogenase 3* [ADH_3 *(2-2)* associated with decreased alcohol catabolism] in relation to risk of colorectal adenoma in the Health Professionals Follow-Up Study. Among 379 cases and 726 controls, *MTHFR* genotypes were not appreciably related to risk of adenoma, but a suggestive interaction ($P = 0.09$) was observed between *MTHFR 677C→T* and alcohol intake; men with *TT* homozygotes who consumed 30+ g/day of alcohol had an odds ratio (OR) of 3.52 [95% confidence interval (CI), 1.41–8.78] relative to drinkers of ≤5 g/day with the *CC/CT* genotypes. ADH_3 genotype alone was not appreciably related to risk, but its influence was modified by alcohol intake. Compared with fast alcohol catabolizers [ADH_3*(1-1)*] with low intakes of alcohol (≤5 g/day), high consumers of alcohol (30+ g/day) had a marked increase in risk if they had the genotype associated with slow catabolism [ADH_3*(2-2)*; OR, 2.94; 95% CI, 1.24–6.92] or intermediate catabolism [ADH_3*(1-2)*] of alcohol (OR, 1.83; 95% CI, 1.03–3.26) but not if they were fast catabolizers [ADH_3*(1-1)*; OR = 1.27; 95% CI = 0.63–2.53). In addition, an increased risk of colorectal adenoma (OR, 17.1; 95% CI, 2.1–137) was observed for those with the ADH_3*(2-2)* genotype and high alcohol-low folate intake compared with those with low alcohol-high folate intake and the ADH_3*(1-1)* genotype (P for interaction = 0.006). Our results indicate that high intake of alcohol is associated with an increased risk of colorectal adenoma, particularly among *MTHFR 677TT* and ADH_3*(2-2)* homozygotes. The findings that alcohol interacts with a folate-related gene (*MTHFR*) and that the interaction between alcohol and ADH_3 is stronger among those with low folate intake support the hypothesis that the carcinogenic influence of alcohol in the large bowel is mediated through folate status.

Introduction

An increased occurrence of colorectal adenomas, precursors of colorectal cancer, is observed fairly consistently among individuals with low intakes (1–5) or circulating levels of folate (2, 6). In case-control and cohort studies of folate intake and levels and colorectal cancer risk, inverse associations were seen in most (1, 7–18), although several studies provided equivocal results (5, 19) or were supportive (20, 21). In addition, almost all of these studies found higher alcohol intake, which antagonizes folate (22), is associated with higher risk of colorectal neoplasia (1–5, 7, 9, 11, 12, 14, 17, 23, 24). The higher risk associated with lower folate or higher alcohol has not always been statistically significant and has been relatively moderate in magnitude. However, a 2–5-fold elevation in colorectal cancer or adenoma risk is relatively consistently observed among individuals with high intake of alcohol and low intake of folate relative to those with low alcohol and high folate intakes (3, 7, 11–14, 17, 18, 24, 25), although some studies do not show this (20, 21). The association has been less clear for women (7, 14, 17, 20, 21) but has been statistically significant in essentially every study that included men (3, 24, 25) or examined men separately (7, 11, 12, 14), possibly because of higher alcohol consumption in men.

The specific mechanisms whereby folate and alcohol influence colorectal carcinogenesis are unclear, but folate is required in the form of 5-methyl-THF[3] to produce methionine required for DNA methylation and as 5,10-methylene THF to convert dUMP into dTMP, a limiting nucleotide for DNA synthesis (Fig. 1). Various experiments indicate that deficiency of folate in either form, leading to abnormalities in DNA synthesis (and repair) or methylation, could enhance carcino-

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[3] The abbreviations used are: THF, tetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; HPFS, Health Professionals Follow-Up Study; OR, odds ratio; CI, confidence interval; BMI, body mass index.

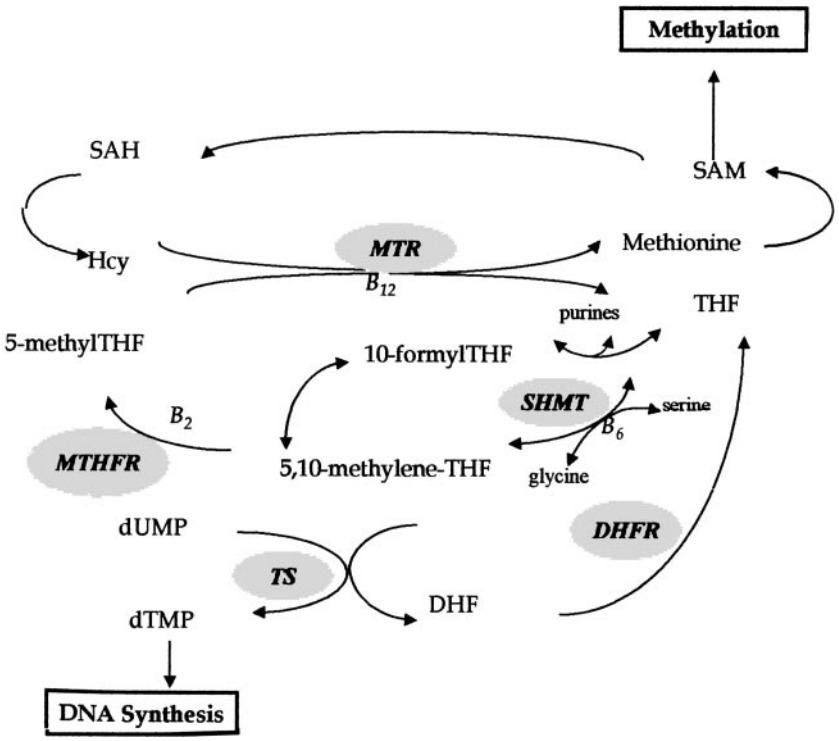

Fig. 1. Competing pathways in folate metabolism. MTHFR = 5,10 methylene THF reductase.

genesis (26). MTHFR is at a critical metabolic branch point that directs the folate pool toward remethylation of homocysteine to methionine at the expense of thymidylate synthesis (Fig. 1). A single nucleotide polymorphism of the *MTHFR* gene (*677C→T*) is associated with an alanine-to-valine substitution and is correlated with enzyme thermolability (27) and reduced enzyme activity (28). *TT* homozygotes tend to accumulate 5,10-methylene THF intracellularly at the expense of 5-methyl THF, whereas individuals with the *CC* or *CT* genotypes have predominantly 5-methyl THF intracellularly (29).

Because deficiencies of either 5-methyl-THF or 5,10-methylene THF could enhance colorectal neoplasia, polymorphic forms of MTHFR may interact with folate and alcohol in influencing risk. Four studies have reported on interactions between folate or alcohol with *MTHFR* and risk of colorectal cancer (16, 30–32) and six have reported on adenoma risk (33–37). In general, among individuals with the *CC* or *CT* genotypes, only modest associations are observed between alcohol and folate and risk of colorectal neoplasia. In contrast, *TT* homozygotes appear to be hyperresponders to folate or alcohol, which are at relatively low risk (compared with those with *CC* or *CT* genotypes) if they have a low-risk diet (high folate and low alcohol) but have no apparent protection or may even have elevated risks if they have a high-risk diet (high alcohol and low folate). This interaction is more striking for alcohol than for folate (16, 30, 35). A second common polymorphism in the COOH-terminal regulatory domain of the *MTHFR* [*1298A→C* (gln→ala)] has been identified (38, 39), yet its function remains controversial. Individuals with combined heterozygosity for *MTHFR 677CT/1298AC* showed reduced enzyme activities, elevated plasma homocysteine, and decreased plasma folate, similar to those with the 677*TT* genotype (39); however, these findings were not reproduced in all studies (40, 41).

The association between alcohol intake and risk of colorectal neoplasia could also be modified by germ-line variants in enzymes that metabolize ethanol. The initial steps in ethanol metabolism, which occur mostly in the liver, are its catabolism to acetaldehyde by ADH followed by the additional breakdown of acetaldehyde to acetate by ALDH (42, 43). Many of the adverse effects of alcohol, particularly both the antifolate and the carcinogenic properties, are attributable to acetaldehyde (44). Variants that influence the conversion rate of ethanol to acetaldehyde have been described for ADH_2 and ADH_3 (42, 43). In Caucasians, a common polymorphism has been described for ADH_3, for which a 2.5-fold higher rate of maximal velocity of ethanol oxidation has been observed for the homodimeric γ1 enzyme relative to the homodimeric γ2 enzyme (45); germ-line variants in ADH_2 are uncommon in Caucasians. Possibly, variation in ADH activity, by determining the rate of ethanol breakdown and acetaldehyde production may influence colorectal carcinogenesis. However, the relationship between human ADH and colorectal neoplasia may be complex because intestinal bacterial ADH activity is probably the major generator of acetaldehyde levels in the large bowel mucosa of alcohol drinkers (46).

To better understand the interrelations among alcohol and folate intakes and related genetic polymorphisms in folate and alcohol metabolism genes, we examined folate, alcohol, and *MTHFR* and ADH_3 polymorphisms in relation to risk of colorectal adenomas in the HPFS.

Materials and Methods

Study Population. The HPFS, an ongoing prospective study of the causes of chronic diseases in men, started in 1986 when 51,529 United States male dentists, optometrists, osteopaths, podiatrists, pharmacists, and veterinarians ages 40–75 years, responded to a mailed questionnaire (47). These men provided baseline information on age, marital status, height and weight, ancestry, medications, smoking history, medical history, phys-

ical activity, and diet. We updated exposure and medical history information every 2 years. Blood samples were provided by 18,018 cohort members from 1993 to 1995. The study was approved by the Institutional Review Boards of the Harvard School of Public Health and Brigham and Women's Hospital.

Assessment of Diet. A semiquantitative food-frequency questionnaire, described in detail previously (48), was administered in 1986, as well as every 4 years thereafter. The questionnaires contained a list of ~130 food and beverage items, each with a specified commonly used unit or portion size, and an open-ended section for unlisted foods. Each participant reported how often, on average, over the past year, he consumed the specified amount of each item and reported on brand of breakfast cereal, and brand, duration, and frequency of vitamin supplements. Nutrient intakes were based on composition values from United States Department of Agriculture sources, supplemented with other data.

The mean correlation coefficients between intakes determined by two 1-week diet records and the dietary questionnaire (adjusting for week-to-week variation in the diet records) among a sample of 127 cohort members were 0.65 for nutrients and 0.63 for specific foods (48, 49). For folate, the correlation coefficient was 0.77. In this sample, total folate intake from food and supplements also correlated with erythrocyte folate levels ($r = 0.56$). The mean erythrocyte folate level (and SE) ranged from 325 ng/ml (± 16) for men in the lowest quintile of intake to 416 ng/ml (± 25) for men in the highest quintile of intake (3). The Spearman correlation between alcohol from dietary records and that from the diet questionnaire was 0.86. Alcohol intake was also related inversely with erythrocyte folate levels; controlling for folate intake by multiple linear regression, each drink of an alcoholic beverage was associated with a reduction of 18.4 ± 7.4 (SE) ng/ml erythrocyte folate ($P = 0.01$). This inverse correlation was primarily attributable to the 17 men who had at least two drinks (~30 g) of alcohol daily, who all had erythrocyte folate levels at or below the median, although their folate intake was comparable with the other men (3).

Identification of Adenoma Cases and Controls. Methods for identifying and confirming adenoma cases have been described in detail previously (3). Briefly, when a participant reported a diagnosis of colorectal polyps on the follow-up questionnaires, we asked him for permission to acquire the relevant medical records. All cases of adenomatous polyps in this analysis were confirmed through histopathological reports reviewed by a study investigator without knowledge of diet and other exposures. On the basis of the endoscopy and pathology reports, we recorded adenoma number, site, size, and histology (tubular, tubulovillous, and villous).

To be eligible for selection as a case or control, a man must have completed a valid dietary questionnaire in 1986, supplied a blood sample between 1993 and 1995, have undergone sigmoidoscopy or colonoscopy after the date of return of the 1986 dietary questionnaire, and not have had a cancer or adenoma diagnosis before the date of endoscopy. Two controls were matched to each case on year of birth, year of endoscopy, and whether they had had a previous endoscopy. We initially identified a total of 370 cases diagnosed through 1994 and 736 matched pair controls. Subsequently, 9 of the controls were identified to be cases, so the final analysis included 379 cases and 727 controls. Among the 379 cases, 79 had at least one adenoma in the proximal colon, 225 in the distal colon, and 95 in the rectum [Note: these numbers sum to >379 because an individual could have an adenoma(s) in more than one subsite.] For the cases, we identified 148 as having large or tubulovillous/villous adenomas.

MTHFR and ADH_3 Genotypes. Genotyping for *MTHFR 677C→T* polymorphism was carried out based on methods described by Chen *et al.* (30). In brief, two primers are designed from the cDNA sequence to generate a 198-bp fragment. The primer sequences are: 5′-TGAAGGAGAAGGTGT-CTGCGGGA-3′ and 5′-AGGACGGTGCGGTGAGAGTG-3′. Amplification was performed using initial denaturation at 95°C for 2 min followed by 29 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s with a final extension at 72°C for 10 min. The buffer for PCR reaction included 20 mM Tris (pH 8.8), 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 7.5 mM $MgSO_4$, and 0.1% Triton X-100. The PCR products were digested with *Hin*fI and size fractionated on 6% polyacrylamide gels. For genotyping for the *MTHFR 1298A→C* polymorphism, two primers were designed from the cDNA sequence to generate a 138-bp fragment (38). The primer sequences are: 5′-GGGAGGAGCTGACCAGTG-CAG-3′ and 5′-GGGGTCAGGCCAGGGGCAG-3′. The PCR products were digested with *Fnu*4HI into 119- and 19-bp fragments and size fractionated on 6% polyacrylamide gels.

Genotyping for ADH_3 was carried out using a PCR-RFLP-based method modified from that of Freudenheim *et al.* (50). In brief, primers with the sequences 5′-GCTAAGAAGT-TTTCACTGGATGC-3′ and 5′-ACCTCTTTCCAGAGCGA-AGC-3′ were used in PCR reactions. Genomic DNA was predigested with *Nla*III for 1.5 h and then amplified with AmpliTaq Gold, using initial denaturation at 95°C for 9 min followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s with a final extension of 72°C for 10 min. PCR products were digested with *Ssp*I and size fractionated on 3.5% low-melting agarose gels.

Laboratory personnel were blind to case-control status. We were unable to genotype 4 men for *MTHFR 677C→T*, 3 men for *MTHFR 1298A→C*, and 2 men for ADH_3. Blinded quality control samples were added for each genotype. The error rate for reproducibility was 0% for the *MTHFR 677* and the ADH_3 polymorphisms and was <5% for the *MTHFR 1298* polymorphism. All three genotypes were in agreement with Hardy-Weinberg equilibrium.

Statistical Analyses. We considered total colorectal adenoma and advanced adenoma (large or tubulovillous/villous) in relation to the genotypes. For *MTHFR 677C→T*, we combined the *CC* and the *CT* genotypes because most previous studies (30, 31, 33–37) generally indicate that these have a similar risk level and because the risk for colorectal adenoma was similar in this population (Table 1). The *MTHFR 1298A→C* polymorphism was categorized into three genotypes, *AA*, *AC*, and *CC*, because functionality of these, if any, is uncertain. We also examined whether the combined heterozygosity of the two *MTHFR* polymorphisms (*677C→T* and *1298A→C*) influenced risk of colorectal adenoma. For ADH_3, we examined the three genotypes ADH_3*(1-1)* (fast metabolizers), ADH_3*(1-2)* (intermediate), and ADH_3*(2-2)* (slow).

We then considered these genotypes in combination with alcohol and folate intakes and for combinations of alcohol and folate. For these analyses, the men were categorized into tertiles of energy-adjusted folate and categories of alcohol intake (<5, 5–30, and >30 g) based on 1986 intakes. When alcohol and folate were combined, high risk was considered as alcohol intakes of ≥20 g/day in combination with folate intake in the low tertile. For the combined analysis, we used 20 g for the cutpoint as done previously (3, 12) because if 30 g was used, the numbers were insufficient. Low risk was considered as

Table 1 ORs of colorectal adenoma associated with *MTHFR 677C→T, 1298A→C*, and combined genotype in the HPFS (1986–1995)

Genotype	All	*MTHFR (1298)*		
		1298AA	*1298AC*	*1298CC*
All		1.0 (ref)	1.09 (0.84–1.43)[a]	0.84 (0.50–1.41)
		[186; 369][b]	[165; 299]	[24; 57]
MTHFR677				
677CC	1.0 (ref)	1.0 (ref)	0.98 (0.62–1.53)	0.75 (0.41–1.37)
	[157; 299]	[51; 92]	[81; 150]	[23; 56]
677CT	0.99 (0.75–1.30)	0.87 (0.56–1.35)	1.04 (0.66–1.62)	
	[168; 325]	[85; 179]	[82; 145]	[0; 1]
677TT	0.93 (0.62–1.39)	0.90 (0.54–1.48)		
	[49; 101]	[48; 98]	[1; 3]	[0; 0]

[a] OR and 95% CI adjusted for matching variables (age, previous endoscopy, year of endoscopy), and smoking history, aspirin use, BMI, physical activity, and intakes of red meat, folate, and alcohol.
[b] Numbers of cases and controls.

alcohol intakes ≤ 5 g/day and folate in the top tertile of intake. Men were jointly categorized by genotype and intake of alcohol, folate, and their combination. We also considered the influence of genotype on risk of adenoma across strata of alcohol and folate intakes and conversely alcohol and folate intakes across strata of genotype. Our primary analysis was based on the 1986 rather than the updated diet because prior analyses suggest folate and alcohol act relatively early in colorectal carcinogenesis (12, 13).

We used both conditional and unconditional logistic regression for the analyses to compute ORs, 95% CIs, tests for trends and interactions, and to control for potentially confounding variables. Although the dataset was initially based on matched cases and controls, some of the stratified analyses required breaking the match. When we used unconditional logistic regression, we controlled for the matching variables, including age, history of previous endoscopy, and year of endoscopy. For all analyses, we also controlled for other risk factors for adenomas, including family history of colorectal cancer, aspirin use, tobacco use (never, current, and past), BMI, and intakes of red meat and methionine. Conditional and unconditional logistic regression yielded very similar results so only those for unconditional logistic regression are presented. For tests for trend, we used the medians of the categories for alcohol and folate modeled as continuous variables (Wald test). To test for interactions between the dietary exposure and genotype, we computed a cross-product term using the ordinal value of genotype [three-category ordinal value for ADH_3 and *MTHFR 1298 (A→C)* and a two-category ordinal term for *MTHFR (677C→T)*] and the medians of the categories for alcohol and folate. *P* for interaction was based on the Wald test for this cross-product term added to the model. All *P*s are based on two-sided tests. All statistical analyses were done using the SAS 6.12 statistical package (SAS Institute).

Results

Diet and Lifestyle Factors. We first examined whether the risk factors for colorectal adenoma were similar in this sample of cases and controls that provided blood samples compared with previous observations in the whole cohort. For total colorectal adenoma, family history of colorectal cancer was associated with increased risk (multivariate OR, 1.67; 95% CI, 1.03–2.69), current smoking (OR, 1.84; 95% CI, 1.09–3.12), and past smoking (OR, 1.49; 95% CI, 1.12–1.96) were associated with an increased risk relative to never smokers, regular aspirin use was associated with lower risk relative to nonusers (OR, 0.72; 95% CI, 0.54–0.96), and alcohol intake was associated with increased risk, although primarily in those drinking 30+ g/day [OR, 1.75; 95% CI, 1.16–2.63, relative to infrequent (<5g/day) drinkers]. As we previously observed (51), physical activity and BMI were not associated with risk of total adenoma, but these were nonsignificantly associated with risk of advanced adenoma (large and/or tubulovillous/villous; OR, 0.66; 95% CI, 0.35–1.22 for high *versus* low METs quintile of activity) and BMI (OR, 1.24; 95% CI, 0.65–2.39 for high *versus* low category of BMI). Folate intake was not related to risk of total adenoma but was inversely associated with advanced adenoma (OR, 0.64; 95% CI, 0.34–1.18 for high *versus* low quintile), although this association was not statistically significant. The risk patterns observed for this sample were largely similar to that for the entire set of HPFS adenoma cases, as reported previously (3, 12, 51–53).

MTHFR Polymorphisms. Table 1 shows the main results for the two *MTHFR* polymorphisms, *677C→T* and *1298A→C* individually and in combination. A formal test based on Terwilliger and Ott (54) revealed that the two polymorphisms were in strong linkage disequilibrium ($P < 0.001$). Only 5 individuals were homozygous variant at one locus and heterozygous at the other (*677TT/1298AC* or *677CT/1298CC*), and no individual was homozygous variant at both loci (*677TT/1298CC*). In regards to risk of adenoma, neither *MTHFR* genotype showed a statistically significant association. In addition, heterozygotes for either *MTHFR* polymorphism individually or both together, were at similar risk as the wild-type homozygotes (*677CC* or *1298AA*).

We next examined each *MTHFR* polymorphism separately in combination with alcohol and folate intakes (Table 2). For alcohol intake, we found a suggestion of an interaction ($P = 0.09$), with the highest risk observed for alcohol drinkers of 30+ g/day who were also *TT* homozygotes. Although the *TT* homozygotes comprised only 14% of the population (controls), a significant trend between alcohol intake and colorectal adenoma risk was observed in these men ($P = 0.03$) but not among men with the *CC* or *CT* genotypes. In an analysis restricted to the *TT* homozygotes, the multivariate OR for ≥30 *versus* ≤5 g alcohol was 4.94; 95% CI, 1.48–16.51. Conversely, when stratified by alcohol intake, *MTHFR TT* homozygotes had a slightly reduced risk if they were low or moderate drinkers but a higher risk if heavy drinkers (OR, 2.70; 95% CI, 0.93–7.87). For folate or combinations of alcohol and folate, *MTHFR* did not show the same pattern as with alcohol (Table 2).

We then examined the *MTHFR 1298A→C* polymorphism

Table 2 Relationships of alcohol, folate and their combinations to colorectal adenoma risk stratified by *MTHFR 677* genotype in the HPFS (1986–1995)

MTHFR 677	≤5	Alcohol (g/day) 5–30	>30
CC/CT OR (95% CI)[a]	1.0 (ref)	1.11 (0.82–1.49)	1.50 (0.97–2.32)
	[123; 272][b]	[149; 280]	[53; 72]
TT OR (95% CI)[a]	0.79 (0.42–1.49)	0.85 (0.48–1.51)	3.52 (1.41–8.78)
	[15; 43]	[20; 50]	[14; 8]
		P for interaction = 0.09	
MTHFR 677	<338	Folate (μg/day) 338–496	≥497
CC/CT OR (95% CI)[a]	1.0	1.14 (0.81–1.61)	1.09 (0.77–1.54)
	[107; 208]	[108; 201]	[110; 215]
TT OR (95% CI)[a]	0.89 (0.46–1.70)	1.01 (0.54–1.88)	1.16 (0.58–2.33)
	[16; 35]	[18; 40]	[15; 26]
		P for interaction = 0.48	
MTHFR 677	Alcohol ≤5 g/day Folate >497 μg/day	Alcohol/folate Middle	Alcohol ≥20 g/day Folate <338 μg/day
CC/CT OR (95% CI)[a]	1.0	0.84 (0.57–1.24)	1.40 (0.77–2.54)
	[48; 92]	[242; 491]	[35; 41]
TT OR (95% CI)[a]	0.45 (0.12–1.69)	0.82 (0.49–1.40)	2.08 (0.61–7.07)
	[3; 13]	[39; 83]	[7; 5]
		P for interaction = 0.20	

[a] OR and 95% CI controlling for matching variables (age, previous endoscopy, year of endoscopy), and smoking history, aspirin use, BMI, physical activity, and intakes of red meat and alcohol or folate.
[b] [cases; controls].

Table 3 Relationships of alcohol and folate intake to colorectal adenoma risk stratified by *MTHFR 1298* genotype in the HPFS (1986–1995)

MTHFR 1298	≤5	Alcohol (g/day) 5–30	>30
1298 AA OR (95% CI)[a]	1.0 (ref)	0.99 (0.66–1.48)	2.36 (1.37–4.07)
	[66; 155][b]	[78; 175]	[42; 39]
1298 AC OR (95% CI)[a]	1.07 (0.72–1.66)	1.33 (0.88–2.03)	1.46 (0.80–2.69)
	[64; 137]	[76; 125]	[25; 37]
1298 CC OR (95% CI)[a]	0.98 (0.42–2.28)	0.94 (0.47–1.91)	0.43 (0.05–4.08)
	[9; 22]	[14; 31]	[1; 4]
		P for interaction = 0.82	
MTHFR 1298	<338	Folate (μg/day) 338–496	≥497
1298 AA OR (95% CI)[a]	1.0	1.43 (0.92–2.23)	1.37 (0.87–2.15)
	[55; 128]	[68; 122]	[63; 119]
1298 AC OR (95% CI)[a]	1.35 (0.83–2.16)	1.40 (0.87–2.25)	1.35 (0.85–2.15)
	[56; 97]	[52; 97]	[57; 105]
1298 CC OR (95% CI)[a]	1.41 (0.61–3.27)	0.82 (0.32–2.07)	0.93 (0.34–2.55)
	[11; 18]	[7; 22]	[6; 17]
		P for interaction = 0.18	

[a] OR and 95% CI controlling for matching variables (age, previous endoscopy, year of endoscopy), and smoking history, aspirin use, BMI, physical activity, and intakes of red meat and alcohol or folate.
[b] [cases; controls].

in combination with alcohol and folate in relation to adenoma risk. Unlike the *MTHFR 677C→T* polymorphism, interactions between alcohol intake and the *MTHFR 1298A→C* polymorphism were not apparent (Table 3). Neither was there an interaction with folate intake or combinations of high alcohol and low folate, although the power was limited (data not shown).

ADH_3 Polymorphism. Compared with the *ADH_3(1-1)* genotype (fast alcohol metabolizers), the multivariate OR, 0.93 (95% CI, 0.70–1.22) for the *ADH_3(1-2)* genotype (intermediate metabolizers) and OR, 0.94 (95% CI, 0.64–1.40) for the *ADH_3(2-2)* genotype (slow metabolizers). We then examined the joint association between *ADH_3* genotype with alcohol intake, folate intake, and with the combination of alcohol and folate intakes (Table 4). Alcohol was not appreciably related to risk of colorectal adenoma among those with the *ADH_3(1-1)* genotype, but consumption of 30+ g of alcohol daily was associated with a higher risk among men with the *ADH_3(1-2)* genotype, and risk was even higher among frequent drinkers with the *ADH_3(2-2)* genotype. The test for interaction for increasing level of alcohol intake across levels (1–3) of *ADH_3* genotype was not significant ($P = 0.17$). Also, among men with the *ADH_3(2-2)* genotype, those in the lowest tertile of folate intake were at higher risk compared with those in the middle or upper tertiles of folate, whereas no clear pattern was apparent

Table 4 Relationships of alcohol, folate and their combinations to colorectal adenoma risk stratified by ADH_3 genotype in the HPFS (1986–1995)

ADH_3	≤5	Alcohol (g/day) 5–30	>30
1–1 OR (95% CI)[a]	1.0 (ref)	1.33 (0.85–2.07)	1.27 (0.63–2.53)
	[55; 127][b]	[72; 116]	[18; 30]
1–2 OR (95% CI)[a]	0.99 (0.64–1.54)	1.02 (0.67–1.57)	1.83 (1.03–3.26)
	[65; 149]	[76; 162]	[34; 39]
2–2 OR (95% CI)[a]	1.06 (0.55–2.02)	0.83 (0.45–1.53)	2.94 (1.24–6.92)
	[18; 40]	[21; 53]	[16; 11]
		P for interaction = 0.17	
ADH_3	<338	Folate (μg/day) 338–496	≥497
1–1 OR (95% CI)[a]	1.0	1.51 (0.90–2.52)	1.32 (0.78–2.23)
	[41; 88]	[56; 91]	[48; 94]
1–2 OR (95% CI)[a]	1.04 (0.63–1.70)	1.18 (0.71–1.95)	1.34 (0.81–2.21)
	[61; 131]	[55; 114]	[59; 104]
2–2 OR (95% CI)[a]	1.97 (0.97–4.02)	0.93 (0.45–1.95)	1.01 (0.52–1.96)
	[21; 24]	[14; 36]	[20; 44]
		P for interaction = 0.55	

ADH_3	Alcohol/Folate	
	Alcohol <20 g/day or Folate ≥338 μg/day	Alcohol ≥20 g/day and Folate <338 μg/day
1–1 OR (95% CI)[a]	1.0	0.65 (0.28–1.49)
	[136; 251]	[9; 22]
1–2 OR (95% CI)[a]	0.85 (0.64–1.13)	1.75 (0.93–3.30)
	[152; 326]	[23; 23]
2–2 OR (95% CI)[a]	0.79 (0.52–1.19)	17.1 (2.13–137.0)
	[45; 103]	[10; 1]
	P for interaction = 0.006	

[a] OR and 95% CI controlling for matching variables (age, previous endoscopy, year of endoscopy), and smoking history, aspirin use, BMI, physical activity, and intakes of red meat and alcohol or folate.
[b] [cases; controls].

for folate intake for the other genotypes (Table 4). In alternative analyses where we stratified the cohort by ADH_3 genotype, alcohol became an increasingly stronger risk factor across the slower metabolizing genotypes [OR for 30+ *versus* <5 g/day alcohol = 1.43; 95% CI, 0.68–3.01) for *ADH_3(1-1)*, (OR, 1.95; 95% CI, 1.09–3.49) for *ADH_3(1-2)*, and OR, 3.14; 95% CI, 0.84–11.76 for *ADH_3(2-2)*]. Across strata of alcohol, the association between ADH_3 and colorectal adenoma risk varied. In never/infrequent drinkers, ADH_3 was unrelated to risk [OR, 1.00; 95% CI, 0.51–1.96 for *ADH_3(2-2) versus ADH_3(1-1)*]; in moderate drinkers having the slower genotype tended to be associated (nonsignificantly) with lower risk (OR, 0.62; 95% CI, 0.34–1.14), and among high drinkers, the slow genotype tended to be associated (nonsignificantly) with greater risk (OR, 2.46; 95% CI, 0.82–7.41).

A marked increased risk of colorectal adenoma (OR, 17.1; 95% CI, 2.1–1.37) was observed for those with the *ADH_3(2-2)* genotype and high alcohol-low folate intake. Although the CIs were wide (2.1–137), the test for interaction for increasing high alcohol and low folate simultaneously across levels of ADH_3 genotype was statistically significant ($P = 0.006$).

Large and Tubulovillous/Villous Adenomas. We conducted all analyses described above for large and tubulovillous/villous adenomas. Although the number of cases in some strata were small and thus confidence intervals were wider, the results were generally in concordance to those with total adenomas (results not shown).

Discussion

Our results show some consistencies with previous studies regarding the *MTHFR 677C→T* polymorphism. In all four studies for cancer (16, 30–32) and for four (33, 35–37) of six (33–37, 55) studies of adenomas, the lowest risk has been associated with the *TT* genotype and low-risk diet (high folate and/or low alcohol). In contrast, among those with low folate or high alcohol intakes, this benefit becomes attenuated, and the risk in the *TT* homozygotes may even exceed that of all others. Thus, *MTHFR TT* homozygotes appear to be hyperresponders to folate status, which are relatively resistant to colorectal neoplasia when folate is high and alcohol is low but are at high risk when folate is low or alcohol is high. In addition, we found that men who have an ADH_3 genotype associated with slower metabolism of alcohol are at considerably higher risk of colorectal adenoma if they consume substantial alcohol and have a low folate diet.

These findings may provide some insights into colorectal carcinogenesis. If folate intake is adequate (and alcohol intake not excessive), *MTHFR 677 TT* homozygotes tend to accumulate appreciable levels of 5,10-methylene THF intracellularly, whereas *CC* and *CT* individuals have 5-methyl THF predominantly (Ref. 29; Fig. 1). The retention of folate in these different forms may have phenotypic advantages or disadvantages that depend on folate status. For example, under conditions of high folate, the *TT* genotype, presumably by allowing the accumulation of folate in the forms required for DNA and RNA synthesis (29), may improve fetal health (56) and hematopoiesis (57). When levels of 5,10-methylene THF are low, misincorporation of uracil for thymidine may occur during DNA synthesis (58), leading to increased frequency of chromosomal breaks (59). Thus, under high folate conditions, the *TT* genotype may be beneficial by enhancing the pool of 5,10-methylene THF. However, when folate is deficient, the *TT* genotype

may be deleterious. Low folate status and the *TT* genotype interact to increase risk of neural tube defects (60, 61) and hyperhomocysteinemia (62), which is a risk factor for cardiovascular disease, and decreases DNA methylation (63), which may enhance carcinogenesis (26, 64). Interestingly, both *TT* genotype and poor folate status appear to be required to adversely influence DNA methylation because those with other genotypes (*CT* or *CC*) do not experience DNA hypomethylation even if they have low folate levels (29). In a recent small study, leukocyte and colonic hypomethylation were associated with a significantly increased risk of adenoma (65).

The haplotype frequency of the *MTHFR 677* and the *1298* polymorphism were examined recently in a meta-analysis of 16 studies that provided reliable data on combined *MTHFR* genotypes in general populations ($n = 5389$; Ref. 66). The percentage for *MTHFR 677/1298* from our population and from (the meta-analysis) are as follows: *CC/AA*, 0.13(0.15); *CC/AC*, 0.21(0.22); *CC/CC*, 0.08(0.085); *CT/AA*, 0.25(0.22); *CT/AC*, 0.20(0.20); *CT/CC*, 0.0018(0.0025); *TT/AA*, 0.135(0.11); *TT/AC*, 0.0041(0.0046); and *TT/CC*, 0(0.00032). Our results, even for the rare haplotypes (*CT/CC*, *TT/AC*, and *TT/CC*), are quite comparable. However, small errors in genotyping can influence the prevalence of the rarer genotypes substantially.

The relation between the *MTHFR 1298A→C* polymorphism and risk of colorectal cancer was investigated in two previous studies. In the Physicians' Health Study (67), which was based on 211 cases and 343 controls, a nonsignificant lower risk was observed with the *1298 CC* genotype (relative risk = 0.73; 95% CI = 0.37–1.43). Moreover, the association was not modified by plasma folate status, but alcohol intake was not examined. A case-control study of African Americans and whites found that the *1298 CC* genotype was inversely associated with risk overall (OR, 0.6; 95% CI, 0.4–0.9) and for whites (OR, 0.5; 95% CI, 0.3–0.9; Ref. 55). Our study did not indicate an appreciable role of the *CC* polymorphism *versus* the *AA* polymorphism in relation to adenoma risk (OR, 0.84; 95% CI, 0.50–1.41), even in combination with folate and alcohol intakes (the OR was ~0.8 compared with *AA/AC* genotype). Because the data are limited, a firm conclusion regarding the *1298 CC* polymorphism cannot be made; the evidence is consistent with no appreciable association or a moderate reduction in risk.

The interaction between the *MTHFR 677C→T* polymorphism and colorectal neoplasia has been striking for alcohol (16, 30, 35). In the current study, the test for interaction was borderline statistically significant ($P = 0.08$), and men with *TT* homozygotes who drank 30+ g alcohol/day had a particularly high risk. In contrast, one study of adenomas found that alcohol increased risk among those with the CC genotype (33). It is striking that four studies found statistically significant or borderline significant interactions with alcohol intake because most studies have had low power to examine this.

Of note, an interaction between *MTHFR 677C→T* with folate in regards to colorectal neoplasia intake has been less evident than the interaction between MTHFR and alcohol. This finding may possibly be related to the relatively good folate status in the United States where most of the studies were conducted. For example, in the current study, very few men had folate intakes < 200 μg, and about half had levels exceeding 400 μg/day, and in a subsample, the mean erythrocyte level was well within the normal range (325 ng/ml) in men in the lowest quintile of folate intake. In contrast, in a study of advanced adenomas in Norway (36), a strong interaction between erythrocyte folate status and *MTHFR* was observed, as well as a 6-fold elevated risk of advanced adenomas among men with *TT* homozygotes with poor folate status relative to *TT* homozygotes with good folate levels. This finding suggests that the potential interaction with folate could possibly be quite strong in populations with poor folate status. In United States studies, an interaction with folate alone, without considering alcohol concurrently, may be difficult to elicit, especially with current fortification with folic acid.

The strong interaction between alcohol and *MTHFR 677C→T* may reflect the potent effects of alcohol on folate metabolism in proliferative tissues such as the intestines and bone marrow (22). Heavy alcohol consumption impairs hematopoiesis, especially in those with poor folate status, causing megaloblastic anemia, as well as neutropenia and thrombocytopenia and can prevent the hematological response to folic acid supplementation in these patients (22). Recent evidence also suggests an interaction between alcohol and the *MTHFR 677C→T* genotype in regards to homocysteine levels. In one study, the prevalence of hyperhomocysteinemia in heavy drinkers was 84.2% in *TT* homozygotes, 54.3% in *CT* heterozygotes, and 31.6% in *CC* homozygotes (68). Among heavy drinkers who were *TT* homozygotes, homocysteine levels were remarkably elevated if folate levels were low (up to 10-fold above normal in some individuals) but were not markedly increased if folate levels were high.

Alcohol's impairment of folate metabolism may be caused by blocking release of folate from the hepatocyte, inhibiting DNA methyltransferase or methionine synthase, trapping folate as 5-methyl THF (thereby depleting cellular 5,10-methylene THF), and inducing intestinal malabsorption of folate (22, 69, 70). Additionally, acetaldehyde at high concentrations cleaves folate at the C9-N10 bond (71), although concentrations of acetaldehyde that far exceed typical concentrations in the blood and most tissues in the body may be required for this effect. However, intestinal bacteria are capable of oxidizing ethanol in the colon to produce substantial levels of acetaldehyde locally (72). In rats, ethanol consumption increases the concentration of acetaldehyde in the colonic mucosa while decreasing the colonic mucosal folate level by 48% (73). The concentrations of acetaldehyde produced by the large bowel bacteria when alcohol is consumed, perhaps 1000-fold higher than circulating levels, may be sufficiently high to break down folate (72, 73). In rats, chronic alcohol consumption induces genomic DNA hypomethylation in the intestinal mucosa (74), which may contribute to carcinogenesis. Although bacterial catabolism of ethanol have been studied mostly in rats, incubation of human colonic contents with various ethanol concentrations *in vitro* at 37°C results in significant production of acetaldehyde (46).

The adverse influence of alcohol on colorectal neoplasia suggests a modifying role of alcohol-metabolizing enzymes. Alcohol is oxidized mainly by ADH into acetaldehyde, which is then catabolized by ALDH (42, 43). A well-known variant of ALDH causes slow breakdown of acetaldehyde and hence high levels of this compound. Interestingly, Japanese alcoholics with the slow-metabolizing ALDH variant have a much higher risk of alcohol-related cancers, including colon cancer (75–77), consistent with an adverse effect of acetaldehyde. The ALDH variant cannot be studied in a predominantly white population such as the HPFS because it occurs exclusively in Asian populations. A common polymorphism in alcohol metabolism identified in Caucasian populations is in ADH_3, where the $\gamma 1$ allele differs from the $\gamma 2$ by two amino acids at positions 271 and 349. Pharmacokinetic studies show a 2.5-higher rate of maximal velocity of ethanol oxidation for the homodimeric $\gamma 1$ compared with homodimeric $\gamma 2$ enzyme (45). Although an influence on short-term blood alcohol levels has not been

clearly demonstrated, studies have associated the *ADH_3* polymorphism with risk of alcoholism (78) and cirrhosis (79). Moreover, moderate alcohol drinkers who are homozygous for the slow-oxidizing *ADH_3* allele have higher high-density lipoprotein cholesterol levels and a lower risk of myocardial infarction (80), suggesting that a lower clearance rate of alcohol enhances the benefit of moderate alcohol intake on high-density lipoprotein cholesterol and coronary disease. The *ADH_3* genotype also appears to modify the response to some plasma steroid hormones to alcohol (81). These relationships suggest that this polymorphism is functional and influences the human physiological response to alcohol consumption.

The relationship of the *ADH_3* genotype to various alcohol-related cancers, including breast (50, 81) and oral-pharyngeal cancer (82, 83), has been equivocal. Only two studies have been reported for colorectal neoplasia (84, 85). The results for *ADH_3* from a study based on 211 incident cases of colorectal cancer and 1113 controls from the Physicians' Health Study were not statistically significant, although there were some limitations (84). The top cutpoint for alcohol consumption was >5 drinks/week, and no overall relationship was observed between alcohol intake and colorectal cancer risk (P trend = 0.25). In the current study, we observed a clear association for alcohol only at levels exceeding 30 g/day (~15 drinks or more/week), which is consistent with most prospective data on alcohol and colorectal cancer. Moreover, in an analysis of colorectal cancer limited to slow metabolizers [*ADH_3(2-2)*] in the Physicians' Health Study, a 3.7-fold increase with higher alcohol consumption was observed (P trend = 0.04), similar to the 3.2-fold increased risk we observed with higher alcohol consumption in the *ADH_3(2-2)* homozygotes. Thus, both studies suggest that slow-metabolizers are more susceptible to the effects of alcohol. In contrast, a case-control study of 433 adenoma cases and 436 polyp-free controls conducted in the Netherlands had apparently opposing results; similar to our study, alcohol increased risk, but risk associated with alcohol appeared to be stronger for men with the *ADH_3(1-1)* genotype than for men with other genotypes (85). However, the interaction term was not statistically significant (P = 0.4). Thus, the limited results from the literature to date are not consistent, and larger additional studies are required to resolve this. A theoretical complexity is that decreased ADH_3 activity may have a dual role for folate in the intestinal mucosa. Lower *ADH_3* activity in the liver could reduce the rate of acetaldehyde production in the liver (beneficial for overall folate status) but could increase ethanol reaching the large intestine, which is then available for acetaldehyde conversion by the microflora (adverse for intestinal folate status).

Several potential limitations of this study should be discussed. Only about one-third of eligible men of the cohort provided blood samples. However, it is unlikely that the genotypes are related to the probability of providing a blood sample. The dietary data were collected prospectively, so bias based on differential reporting between cases and controls is unlikely. The major findings we have reported in the overall cohort were similar in this nested, case-control study. Our dietary questionnaire was reasonably valid, although measurement error would be unrelated to genotype and tend only to attenuate true associations. It is possible that some of the heavy drinkers (>30g/day) could be underreporting this alcohol consumption, so it is plausible that risk begins to increase at some level > 30 g/day. The relatively homogeneous nature of the highly educated study population helps ensure valid information and reduces the likelihood of residual confounding by factors related to socioeconomic status but may limit generalizability of findings. An important limitation is that the sample size was relatively small, especially to examine interactions. The statistically significant or near significant interactions are even more impressive in this regard, but the null results (*e.g.*, for *MTHFR 1298 A→C* polymorphism) should be interpreted cautiously because of our limited power.

Another limitation is that few in this population would be expected to suffer from very poor folate status. However, this limitation would tend to diminish our ability to observe associations, and we may have underestimated the full influence of these polymorphisms among those with poor folate status. These results may be most generalizable to the United States, where high rates of vitamin supplementation (and more recently fortification with folic acid) has made severe folate deficiency rare (86). Of note, in a sample from this cohort, alcohol intake was a stronger predictor of low erythrocyte folate levels than dietary folate itself (3), suggesting a prominent role of alcohol in generally folate-adequate populations. Finally, data on *MTHFR* and colorectal neoplasia are relatively sparse for women, so these findings need to be replicated in women.

In conclusion, our results support the hypothesis that high intakes of alcohol, at least two drinks daily, are associated with an increased risk of colorectal adenoma, particularly among *MTHFR 677TT* homozygotes and among slow metabolizers of ethanol based on *ADH_3* genotypes. The findings that alcohol interacts with a folate-related enzyme (MTHFR) and that the interaction between alcohol and *ADH_3* genotype is stronger among those with low folate intakes support the hypothesis that the carcinogenic effect of alcohol in the large bowel is mediated through its adverse effects on folate status.

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